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(81) États désignés (sauf indication contraire, pour tout titre de protection nationale disponible) : AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) États désignés (sauf indication contraire, pour tout titre de protection régionale disponible) : ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), eurasién (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), européen (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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— avec rapport de recherche internationale

[Suite sur la page suivante]

(54) Title: METHOD FOR THE PRODUCTION OF EVOLVED MICROORGANISMS WHICH PERMIT THE GENERATION OR MODIFICATION OF METABOLIC PATHWAYS

(54) Titre : PROCEDE DE PREPARATION DE MICROORGANISMES EVOLUES PERMETTANT LA CREATION OU LA MODIFICATION DE VOIES METABOLIQUES

(57) Abstract: The invention relates to a novel method for the preparation of evolved microorganisms which permit a modification of metabolic pathways, characterised in comprising the following steps: a) production of a modified microorganism by genetic modification of initial microorganism cells such as to inhibit the production of or the consumption of a metabolite when the microorganism is cultivated in a defined medium which also affects the capacity of the microorganism for growth, b) culture of the modified microorganisms previously obtained in said defined medium to induce evolution in said cells where it might be necessary to add a co-substrate to the defined medium in order to permit said evolution, c) selection of the cells of modified microorganisms which are capable of developing in the defined medium, optionally with a co-substrate. The invention also relates to the strains of evolved microorganisms obtained thus, the genes evolved which code for evolved proteins which may be obtained by means of said method and the use of said evolved microorganisms, genes or proteins in a biotransformation method.

(57) Abrégé : La présente invention concerne un nouveau procédé de préparation de microorganismes évolués, permettant une modification des voies métaboliques, caractérisé en ce qu'il comprend les étapes suivantes : a) obtention d'un microorganisme modifié par modification génétique des cellules d'un microorganisme initial de manière à inhiber la production ou la consommation d'un métabolite lorsque le microorganisme est cultivé sur un milieu défini, affectant ainsi la capacité de croissance du microorganisme, b) culture des microorganismes modifiés précédemment obtenus sur ledit milieu défini pour le faire évoluer, il peut être nécessaire d'ajouter un co-substrat au milieu défini afin de permettre cette évolution, a) sélection des cellules de microorganismes modifiés capables de se développer sur le milieu défini, éventuellement avec un co-substrat. L'invention concerne également les souches de microorganismes évolués ainsi obtenus, les gènes évolués codant pour des protéines évoluées susceptibles d'être obtenues par le procédé selon l'invention et l'utilisation desdits microorganismes, gènes ou protéines évolués dans un procédé de biotransformation.

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— avant l'expiration du délai prévu pour la modification des revendications, sera republiée si des modifications sont reçues

(88) Date de publication du rapport de recherche internationale: 16 décembre 2004

En ce qui concerne les codes à deux lettres et autres abréviations, se référer aux "Notes explicatives relatives aux codes et abréviations" figurant au début de chaque numéro ordinaire de la Gazette du PCT.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/FR2004/000354

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/01 C12N9/10 C12P13/06 C12P13/08 C12P13/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, FSTA, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|--|
| X Y | WO 93/17112 A (GENENCOR INT) 2 September 1993 (1993-09-02) cited in the application the whole document | 1-4, 7-17,29, 30,35 18-24, 27,28, 31-34 |
| X Y | WO 02/051231 A (SCHROEDER HARTWIG ;BASF AG (DE); POMPEJUS MARKUS (DE); ZELDER OSKA) 4 July 2002 (2002-07-04) page 38, line 37 - page 40, line 17 page 62, line 29 - page 63, line 15; example 7 ----- -/-- | 1-4, 7-17,29, 30,35 18-24, 27,28, 31-34 |



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

6 September 2004

Date of mailing of the international search report

04 11. 2004

Name and mailing address of the ISA

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/FR2004/000354

| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT | | |
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| X | FLAVIN M ET AL: "ENZYMATIC SYNTHESIS OF HOMOCYSTEINE OR METHIONINE DIRECTLY FROM O-SUCCINYL-HOMOSERINE" BIOCHIMICA ET BIOPHYSICA ACTA, AMSTERDAM, NL, vol. 132, no. 2, 15 March 1967 (1967-03-15), pages 400-405, XP008028828 ISSN: 0006-3002 cited in the application | 11-24, 27,28,35 |
| Y | the whole document | 18-24, 27,28 |
| X | ----- SMITH I K ET AL: "UTILIZATION OF S-METHYLCYSTEINE AND METHYLMERCAPTAN BY METHIONINELESS MUTANTS OF NEUROSPORA AND THE PATHWAY OF THEIR CONVERSION TO METHIONINE. II. ENZYME STUDIES" BIOCHIMICA ET BIOPHYSICA ACTA, AMSTERDAM, NL, vol. 184, no. 1, 17 June 1969 (1969-06-17), pages 130-138, XP008028840 ISSN: 0006-3002 cited in the application | 11-17, 29-35 |
| Y | the whole document | 31-34 |
| X | ----- MAY OLIVER ET AL: "Inverting enantioselectivity by directed evolution of hydantoinase for improved production of L-methionine" NATURE BIOTECHNOLOGY, NATURE PUBLISHING, US, vol. 18, no. 3, March 2000 (2000-03), pages 317-320, XP002154849 ISSN: 1087-0156 the whole document | 1-4,7, 9-16,35 |
| A | ----- WO 02/083892 A (MARLIERE PHILIPPE ; POCHET SYLVIE (FR); BOUZON MADELEINE (FR); CENTRE) 24 October 2002 (2002-10-24) cited in the application claims 1-30 | 1,2, 7-11, 13-16,35 |
| A | ----- DUCHANGE N ET AL: "E.coli metB and metL (5' end) genes coding for cystathione gamma-synthase and aspartokinase II-homoserine dehydrogenase II" EMBL, 13 June 1985 (1985-06-13), XP002274156 the whole document | 16-28 |
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INTERNATIONAL SEARCH REPORT

 International Application No
 PCT/FR2004/000354

| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT | | |
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| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| X | KAWASHIMA T ET AL: "Cystathionine beta lyase / O-succinylhomoserine lyase" EMBL, 1 October 2001 (2001-10-01), XP002274157 the whole document | 16-25, 27,28 |
| A | ----- ARNOLD F H ET AL: "OPTIMIZING INDUSTRIAL ENZYMES BY DIRECTED EVOLUTION" ADVANCES IN BIOCHEMICAL ENGINEERING, BIOTECHNOLOGY, SPRINGER, BERLIN, DE, vol. 58, 1997, pages 1-14, XP000891392 ISSN: 0724-6145 the whole document | 1-11 |
| A | ----- WEISSBACH H ET AL: "REGULATION OF METHIONINE SYNTHESIS IN ESCHERICHIA COLI" MOLECULAR MICROBIOLOGY, BLACKWELL SCIENTIFIC, OXFORD, GB, vol. 5, no. 7, 1987, pages 1593-1597, XP001037155 ISSN: 0950-382X cited in the application the whole document | 1-4,7-35 |
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| A | ----- WO 02/18613 A (DEGUSSA) 7 March 2002 (2002-03-07) the whole document | 1-4,7-35 |
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/FR2004/000354

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| X | KASE H ET AL: "L-METHIONINE PRODUCTION BY METHIONINE ANALOG-RESISTANT MUTANTS OF CORYNEBACTERIUM GLUTAMICUM" AGRICULTURAL AND BIOLOGICAL CHEMISTRY, JAPAN SOC. FOR BIOSCIENCE, BIOTECHNOLOGY AND AGROCHEM. TOKYO, JP, vol. 39, no. 1, 1975, pages 153-160, XP009012948 ISSN: 0002-1369 the whole document ----- | 1-4 |
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| X | NAKAMORI S ET AL: "Mechanism of L-methionine overproduction by Escherichia coli: The replacement of Ser-54 by Asn in the MetJ protein causes the derepression of L-methionine biosynthetic enzymes" APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, SPRINGER VERLAG, BERLIN, DE, vol. 52, no. 2, August 1999 (1999-08), pages 179-185, XP002242975 ISSN: 0175-7598 the whole document ----- | 11-16,35 |

Continuation of Box III

The International Searching Authority has determined that this international application contains multiple (groups of) inventions, as follows:

1. Claims 1-4 and 7-35 (in part, where applicable)

Method for preparing evolved microorganisms allowing modification of a biosynthesis pathway of methionine; microorganisms, genes and proteins obtained by said method; and the use thereof in a biotransformation process.

2. Claims 1-4 and 7-35 (in part, where applicable)

Method for preparing evolved microorganisms allowing modification of a biosynthesis pathway of cysteine; microorganisms, genes and proteins obtained by said method; and the use thereof in a biotransformation process.

3. Claims 1-4 and 7-35 (in part, where applicable)

Method for preparing evolved microorganisms allowing modification of a biosynthesis pathway of threonine; microorganisms, genes and proteins obtained by said method; and the use thereof in a biotransformation process.

4. Claims 1-4 and 7-35 (in part, where applicable)

Method for preparing evolved microorganisms allowing modification of a biosynthesis pathway of lysine; microorganisms, genes and proteins obtained by said method; and the use thereof in a biotransformation process.

5. Claims 1-4 and 7-35 (in part, where applicable)

Method for preparing evolved microorganisms allowing modification of a biosynthesis pathway of isoleucine; microorganisms, genes and proteins obtained by said method; and the use thereof in a biotransformation process.

6. Claims 1, 2 and 7-35 (in part, where applicable)

Method for preparing evolved microorganisms allowing modification of a biosynthesis pathway of nucleic acids; microorganisms, genes and proteins obtained by said method; and the use thereof in a biotransformation process.

7. Claims 1, 2 and 7-35 (in part, where applicable)

Method for preparing evolved microorganisms allowing modification of a biosynthesis pathway of lipids; microorganisms, genes and proteins obtained by said method; and the use thereof in a biotransformation process.

8. Claims 1, 2 and 7-35 (in part, where applicable)

Method for preparing evolved microorganisms allowing modification of a biosynthesis pathway of sugars; microorganisms, genes and proteins obtained by said method; and the use thereof in a biotransformation process.

9. Claims 1, 2 and 7-35 (in part, where applicable); claims 5, 6, 36 and 37 (in full)

Method for preparing evolved microorganisms allowing modification of the metabolic pathways involved in NADPH consumption; microorganisms, genes and proteins obtained by said method; and the use thereof in a biotransformation process.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/FR2004/000354

| Patent document cited in search report | | Publication date | Patent family member(s) | Publication date |
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RAPPORT DE RECHERCHE INTERNATIONALE

Demande Internationale No

PCT/FR2004/000354

A. CLASSEMENT DE L'OBJET DE LA DEMANDE

CIB 7 C12N15/01 C12N9/10 C12P13/06 C12P13/08 C12P13/12

Selon la classification internationale des brevets (CIB) ou à la fois selon la classification nationale et la CIB

B. DOMAINES SUR LESQUELS LA RECHERCHE A PORTE

Documentation minimale consultée (système de classification suivi des symboles de classement)

CIB 7 C12N C12P

Documentation consultée autre que la documentation minimale dans la mesure où ces documents relèvent des domaines sur lesquels a porté la recherche

Base de données électronique consultée au cours de la recherche internationale (nom de la base de données, et si réalisable, termes de recherche utilisés)

EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, FSTA, CHEM ABS Data

C. DOCUMENTS CONSIDERES COMME PERTINENTS

| Catégorie * | Identification des documents cités, avec, le cas échéant, l'indication des passages pertinents | no. des revendications visées |
|-------------|--|-------------------------------|
| X | WO 93/17112 A (GENENCOR INT) 2 septembre 1993 (1993-09-02) cité dans la demande | 1-4, 7-17, 29, 30, 35 |
| Y | le document en entier | 18-24, 27, 28, 31-34 |
| X | WO 02/051231 A (SCHROEDER HARTWIG ; BASF AG (DE); POMPEJUS MARKUS (DE); ZELDER OSKA) 4 juillet 2002 (2002-07-04) | 1-4, 7-17, 29, 30, 35 |
| Y | page 38, ligne 37 - page 40, ligne 17 | 18-24, 27, 28, 31-34 |
| | page 62, ligne 29 - page 63, ligne 15; exemple 7 | |
| | ----- -/- | |

☒ Voir la suite du cadre C pour la fin de la liste des documents

☒ Les documents de familles de brevets sont indiqués en annexe

* Catégories spéciales de documents cités:

"A" document définissant l'état général de la technique, non considéré comme particulièrement pertinent

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"X" document particulièrement pertinent; l'invention revendiquée ne peut être considérée comme nouvelle ou comme impliquant une activité inventive par rapport au document considéré isolément

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"&" document qui fait partie de la même famille de brevets

Date à laquelle la recherche internationale a été effectivement achevée

6 septembre 2004

Date d'expédition du présent rapport de recherche internationale

04.11.2004

Nom et adresse postale de l'administration chargée de la recherche internationale

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Fonctionnaire autorisé

Devijver, K

RAPPORT DE RECHERCHE INTERNATIONALE

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C.(suite) DOCUMENTS CONSIDERES COMME PERTINENTS

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| X | FLAVIN M ET AL: "ENZYMATIC SYNTHESIS OF HOMOCYSTEINE OR METHIONINE DIRECTLY FROM O-SUCCINYL-HOMOSERINE" BIOCHIMICA ET BIOPHYSICA ACTA, AMSTERDAM, NL, vol. 132, no. 2, 15 mars 1967 (1967-03-15), pages 400-405, XP008028828 ISSN: 0006-3002 cité dans la demande | 11-24, 27,28,35 |
| Y | le document en entier | 18-24, 27,28 |
| X | ----- SMITH I K ET AL: "UTILIZATION OF S-METHYLCYSTEINE AND METHYLMERCAPTAN BY METHIONINELESS MUTANTS OF NEUROSPORA AND THE PATHWAY OF THEIR CONVERSION TO METHIONINE. II. ENZYME STUDIES" BIOCHIMICA ET BIOPHYSICA ACTA, AMSTERDAM, NL, vol. 184, no. 1, 17 juin 1969 (1969-06-17), pages 130-138, XP008028840 ISSN: 0006-3002 cité dans la demande | 11-17, 29-35 |
| Y | le document en entier | 31-34 |
| X | ----- MAY OLIVER ET AL: "Inverting enantioselectivity by directed evolution of hydantoinase for improved production of L-methionine" NATURE BIOTECHNOLOGY, NATURE PUBLISHING, US, vol. 18, no. 3, mars 2000 (2000-03), pages 317-320, XP002154849 ISSN: 1087-0156 le document en entier | 1-4,7, 9-16,35 |
| A | ----- WO 02/083892 A (MARLIERE PHILIPPE ; POCHET SYLVIE (FR); BOUZON MADELEINE (FR); CENTRE) 24 octobre 2002 (2002-10-24) cité dans la demande revendications 1-30 | 1,2, 7-11, 13-16,35 |
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| A | ARNOLD F H ET AL: "OPTIMIZING INDUSTRIAL ENZYMES BY DIRECTED EVOLUTION" ADVANCES IN BIOCHEMICAL ENGINEERING, BIOTECHNOLOGY, SPRINGER, BERLIN, DE, vol. 58, 1997, pages 1-14, XP000891392 ISSN: 0724-6145 le document en entier ----- | 1-11 |
| A | WEISSBACH H ET AL: "REGULATION OF METHIONINE SYNTHESIS IN ESCHERICHIA COLI" MOLECULAR MICROBIOLOGY, BLACKWELL SCIENTIFIC, OXFORD, GB, vol. 5, no. 7, 1987, pages 1593-1597, XP001037155 ISSN: 0950-382X cité dans la demande le document en entier ----- | 1-4,7-35 |
| A | DATABASE WPI Section Ch, Week 200043 Derwent Publications Ltd., London, GB; Class B05, AN 2000-485354 XP002265367 & JP 2000 157267 A (AJINOMOTO KK) 13 juin 2000 (2000-06-13) cité dans la demande abrégé ----- | 1-4,7-35 |
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| X | EP 0 519 113 A (AJINOMOTO KK) 23 décembre 1992 (1992-12-23) exemples 1,3 ----- | 1-4 |
| X | US 3 071 518 A (RAFELSON JR MAX E ET AL) 1 janvier 1963 (1963-01-01) colonne 2, ligne 55 - ligne 63 colonne 3, ligne 3 - ligne 6 ----- | 1-4 |
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| Catégorie * | Identification des documents cités, avec, le cas échéant, l'indication des passages pertinents | no. des revendications visées |
| X | <p>KASE H ET AL: "L-METHIONINE PRODUCTION BY METHIONINE ANALOG-RESISTANT MUTANTS OF CORYNEBACTERIUM GLUTAMICUM"</p> <p>AGRICULTURAL AND BIOLOGICAL CHEMISTRY, JAPAN SOC. FOR BIOSCIENCE, BIOTECHNOLOGY AND AGROCHEM. TOKYO, JP, vol. 39, no. 1, 1975, pages 153-160, XP009012948</p> <p>ISSN: 0002-1369</p> <p>le document en entier</p> <p>-----</p> | 1-4 |
| X | <p>GB 2 075 055 A (AJINOMOTO KK)</p> <p>11 novembre 1981 (1981-11-11)</p> <p>exemples 1,5</p> <p>-----</p> | 11-16,35 |
| X | <p>NAKAMORI S ET AL: "Mechanism of L-methionine overproduction by Escherichia coli: The replacement of Ser-54 by Asn in the MetJ protein causes the derepression of L-methionine biosynthetic enzymes"</p> <p>APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, SPRINGER VERLAG, BERLIN, DE, vol. 52, no. 2, août 1999 (1999-08), pages 179-185, XP002242975</p> <p>ISSN: 0175-7598</p> <p>le document en entier</p> <p>-----</p> | 11-16,35 |

RAPPORT DE RECHERCHE INTERNATIONALE

Demande internationale n°
PCT/FR2004/000354

Cadre II Observations - lorsqu'il a été estimé que certaines revendications ne pouvaient pas faire l'objet d'une recherche (suite du point 2 de la première feuille)

Conformément à l'article 17.2)a), certaines revendications n'ont pas fait l'objet d'une recherche pour les motifs suivants:

1. ☐ Les revendications n^{os} se rapportent à un objet à l'égard duquel l'administration n'est pas tenue de procéder à la recherche, à savoir:
2. ☐ Les revendications n^{os} se rapportent à des parties de la demande internationale qui ne remplissent pas suffisamment les conditions prescrites pour qu'une recherche significative puisse être effectuée, en particulier:
3. ☐ Les revendications n^{os} sont des revendications dépendantes et ne sont pas rédigées conformément aux dispositions de la deuxième et de la troisième phrases de la règle 6.4.a).

Cadre III Observations - lorsqu'il y a absence d'unité de l'invention (suite du point 3 de la première feuille)

L'administration chargée de la recherche internationale a trouvé plusieurs inventions dans la demande internationale, à savoir:

voir feuille supplémentaire

1. ☐ Comme toutes les taxes additionnelles ont été payées dans les délais par le déposant, le présent rapport de recherche internationale porte sur toutes les revendications pouvant faire l'objet d'une recherche.
2. ☐ Comme toutes les recherches portant sur les revendications qui s'y prêtaient ont pu être effectuées sans effort particulier justifiant une taxe additionnelle, l'administration n'a sollicité le paiement d'aucune taxe de cette nature.
3. ☐ Comme une partie seulement des taxes additionnelles demandées a été payée dans les délais par le déposant, le présent rapport de recherche internationale ne porte que sur les revendications pour lesquelles les taxes ont été payées, à savoir les revendications n^{os}
4. ☒ Aucune taxe additionnelle demandée n'a été payée dans les délais par le déposant. En conséquence, le présent rapport de recherche internationale ne porte que sur l'invention mentionnée en premier lieu dans les revendications; elle est couverte par les revendications n^{os} 1-4, 7-35 (partiellement, pour autant qu'applicables)

Remarque quant à la réserve

- ☐ Les taxes additionnelles étaient accompagnées d'une réserve de la part du déposant.
- ☐ Le paiement des taxes additionnelles n'était assorti d'aucune réserve.

SUITE DES RENSEIGNEMENTS INDICUES SUR PCT/ISA/ 210

L'administration chargée de la recherche internationale a trouvé plusieurs (groupes d') inventions dans la demande internationale, à savoir:

1. revendications: 1-4,7-35 (partiellement, pour autant qu'applicables)

Procédé de préparation de microorganismes évolués permettant une modification d'une voie de biosynthèse de méthionine, microorganismes, gènes et protéines obtenus par ce procédé et leur utilisation dans un procédé de biotransformation.

2. revendications: 1-4,7-35 (partiellement, pour autant qu'applicables)

Procédé de préparation de microorganismes évolués permettant une modification d'une voie de biosynthèse de cystéine, microorganismes, gènes et protéines obtenus par ce procédé et leur utilisation dans un procédé de biotransformation.

3. revendications: 1-4,7-35 (partiellement, pour autant qu'applicables)

Procédé de préparation de microorganismes évolués permettant une modification d'une voie de biosynthèse de thréonine, microorganismes, gènes et protéines obtenus par ce procédé et leur utilisation dans un procédé de biotransformation.

4. revendications: 1-4,7-35 (partiellement, pour autant qu'applicables)

Procédé de préparation de microorganismes évolués permettant une modification d'une voie de biosynthèse de lysine, microorganismes, gènes et protéines obtenus par ce procédé et leur utilisation dans un procédé de biotransformation.

5. revendications: 1-4,7-35 (partiellement, pour autant qu'applicables)

Procédé de préparation de microorganismes évolués permettant une modification d'une voie de biosynthèse d'isoleucine, microorganismes, gènes et protéines obtenus par ce procédé et leur utilisation dans un procédé de biotransformation.

6. revendications: 1,2,7-35 (partiellement, pour autant qu'applicables)

SUIITE DES RENSEIGNEMENTS INDIQUES SUR PCT/ISA/ 210

Procédé de préparation de microorganismes évolués permettant une modification des voies de biosynthèse des acides nucléiques, microorganismes, gènes et protéines obtenus par ce procédé et leur utilisation dans un procédé de biotransformation.

7. revendications: 1,2,7-35 (partiellement, pour autant qu'applicables)

Procédé de préparation de microorganismes évolués permettant une modification des voies de biosynthèse des lipides, microorganismes, gènes et protéines obtenus par ce procédé et leur utilisation dans un procédé de biotransformation.

8. revendications: 1,2,7-35 (partiellement, pour autant qu'applicables)

Procédé de préparation de microorganismes évolués permettant une modification des voies de biosynthèse des sucres, microorganismes, gènes et protéines obtenus par ce procédé et leur utilisation dans un procédé de biotransformation.

9. revendications: 1,2,7-35 (partiellement, pour autant qu'applicables);
5,6,36,37 (complètement)

Procédé de préparation de microorganismes évolués permettant une modification des voies métaboliques qui participent à la consommation du NADPH, microorganismes, gènes et protéines obtenus par ce procédé et leur utilisation dans un procédé de biotransformation.

RAPPORT DE RECHERCHE INTERNATIONALE

Renseignements relatifs a nbres de familles de brevets

Demande internationale No

PCT/FR2004/000354

| Document brevet cité au rapport de recherche | Date de publication | Membre(s) de la famille de brevet(s) | Date de publication |
|---|------------------------|--|--|
| WO 9317112 A | 02-09-1993 | CA 2130347 A1 EP 0630406 A1 JP 7503855 T WO 9317112 A1 | 02-09-1993 28-12-1994 27-04-1995 02-09-1993 |
| WO 02051231 A | 04-07-2002 | WO 02051231 A1 EP 1345714 A1 JP 2004524827 T US 2004043953 A1 | 04-07-2002 24-09-2003 19-08-2004 04-03-2004 |
| WO 02083892 A | 24-10-2002 | FR 2823219 A1 CA 2444026 A1 EP 1383890 A2 WO 02083892 A2 JP 2004528844 T | 11-10-2002 24-10-2002 28-01-2004 24-10-2002 24-09-2004 |
| JP 2000157267 A | 13-06-2000 | AUCUN | |
| WO 0218613 A | 07-03-2002 | DE 10109690 A1 AU 8966601 A WO 0218613 A1 EP 1313871 A1 US 2002110878 A1 | 14-03-2002 13-03-2002 07-03-2002 28-05-2003 15-08-2002 |
| EP 0519113 A | 23-12-1992 | EP 0519113 A1 | 23-12-1992 |
| US 3071518 A | 01-01-1963 | AUCUN | |
| GB 2075055 A | 11-11-1981 | JP 56144092 A FR 2480306 A1 | 10-11-1981 16-10-1981 |

Translation

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

| | | | |
|--|---|---|-----------------------|
| Applicant's or agent's file reference | FOR FURTHER ACTION | | See Form PCT/IPEA/416 |
| International application No. PCT/FR2004/000354 | International filing date (day/month/year) 17.02.2004 | Priority date (day/month/year) 18.02.2003 | |
| International Patent Classification (IPC) or national classification and IPC C12N15/01 , C12N9/10, C12P13/06, C12P13/08, C12P13/12 | | | |
| Applicant METABOLIC EXPLORER | | | |

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of **11** sheets, including this cover sheet.

3. This report is also accompanied by ANNEXES, comprising:

a. ☐ (sent to the applicant and to the International Bureau) a total of _____ sheets, as follows:

☐ sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).

☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.

b. ☐ (sent to the International Bureau only) a total of (indicate type and number of electronic carrier(s)) _____, containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

| | | |
|-------------------------------------|--------------|---|
| <input checked="" type="checkbox"/> | Box No. I | Basis of the report |
| <input type="checkbox"/> | Box No. II | Priority |
| <input checked="" type="checkbox"/> | Box No. III | Non-establishment of opinion with regard to novelty, inventive step and industrial applicability |
| <input checked="" type="checkbox"/> | Box No. IV | Lack of unity of invention |
| <input checked="" type="checkbox"/> | Box No. V | Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement |
| <input type="checkbox"/> | Box No. VI | Certain documents cited |
| <input type="checkbox"/> | Box No. VII | Certain defects in the international application |
| <input type="checkbox"/> | Box No. VIII | Certain observations on the international application |

| | |
|---|-----------------------------------|
| Date of submission of the demand | Date of completion of this report |
| Name and mailing address of the IPEA/EP | Authorized officer |
| Facsimile No. | Telephone No. |

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.

PCT/FR2004/000354

Box No. I

Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.
- ☐ This report is based on translations from the original language into the following language _____, which is the language of a translation furnished for the purposes of:
- ☐ international search (Rule 12.3 and 23.1(b))
- ☐ publication of the international application (Rule 12.4)
- ☐ international preliminary examination (Rule 55.2 and/or 55.3)
2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:
- ☐ the international application as originally filed/furnished
- ☒ the description:
- pages 1-79 as originally filed/furnished
- pages* _____ received by this Authority on _____
- pages* _____ received by this Authority on _____
- ☒ the claims:
- nos. 1-37 as originally filed/furnished
- nos.* _____ as amended (together with any statement) under Article 19
- nos.* _____ received by this Authority on _____
- nos.* _____ received by this Authority on _____
- ☒ the drawings:
- sheets 1/2-12/12 as originally filed/furnished
- sheets* _____ received by this Authority on _____
- sheets* _____ received by this Authority on _____
- ☒ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.
3. ☐ The amendments have resulted in the cancellation of:
- ☐ the description, pages _____
- ☐ the claims, nos. _____
- ☐ the drawings, sheets/figs _____
- ☐ the sequence listing (*specify*): _____
- ☐ any table(s) related to sequence listing (*specify*): _____
4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
- ☐ the description, pages _____
- ☐ the claims, nos. _____
- ☐ the drawings, sheets/figs _____
- ☐ the sequence listing (*specify*): _____
- ☐ any table(s) related to sequence listing (*specify*): _____

* If item 4 applies, some or all of those sheets may be marked "superseded."

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.

PCT/FR2004/000354

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application

☒ claims Nos. 1-4, 7-35 (in part, where applicable); 5, 6, 36, 37 (in full)

because:

☐ the said international application, or the said claims Nos. _____
relate to the following subject matter which does not require an international preliminary examination (*specify*):

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. see below
are so unclear that no meaningful opinion could be formed (*specify*):

11-28, 35 (in part, where applicable); 29-34 (in full)

See supplemental box

☒ the claims, or said claims Nos. 11-28, 35 (in part, where applicable); 29-34 (in full) are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. 1-4, 7-35 (in part, where applicable); 5, 6, 36, 37 (in full)

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form

☐

has not been furnished

☐

does not comply with the standard

the computer readable form

☐

has not been furnished

☐

does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-bis of the Administrative Instructions.

☐ See Supplemental Box for further details.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.

PCT/FR2004/000354

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation to restrict or pay additional fees the applicant has:
 - ☐ restricted the claims.
 - ☐ paid additional fees.
 - ☐ paid additional fees under protest.
 - ☒ neither restricted the claims nor paid additional fees.
2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is:
 - ☐ complied with.
 - ☒ not complied with for the following reasons:

See supplemental box
4. Consequently, this report has been established in respect of the following parts of the international application:
 - ☐ all parts.
 - ☒ the parts relating to claims Nos. 1-4, 7-35 (in part, where applicable)

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.

PCT/FR2004/000354

| Box No. V | Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement | | |
|--|---|-----------------------|-----|
| 1. Statement | | | |
| Novelty (N) | Claims | 26 | YES |
| | Claims | 1-4, 7-25, 27, 28, 35 | NO |
| Inventive step (IS) | Claims | 26 | YES |
| | Claims | 1-4, 7-25, 27, 28, 35 | NO |
| Industrial applicability (IA) | Claims | 1-4, 7-28, 35 | YES |
| | Claims | | NO |
| 2. Citations and explanations (Rule 70.7) | | | |
| <p>1. Reference is made to the following documents in the present notification:</p> <p>D1: WO 93/17112 A (GENENCOR INT) 2 September 1993 (1993-09-02)</p> <p>D2: DUCHANGE N ET AL: "E. coli metB and metL (5' end) genes coding for cystathione gamma-synthase and aspartokinase II-homoserine dehydrogenase II" EMBL, 13 June 1985 (1985-06-13), XP002274156</p> <p>D3: KAWASHIMA T ET AL: "Cystathionine beta lyase / O-succinylhomoserine lyase" EMBL, 1 October 2001 (2001-10-01), XP002274157</p> <p>2. NOVELTY (PCT Article 33(2)) AND INVENTIVE STEP (PCT Article 33(3))</p> <p>2.1 Document D1 describes (cf. pages 1, 2, 5 (point 5) and examples 1 and 3) a method for preparing evolved microorganisms (<i>E. coli</i>, <i>C. glutamicum</i> and <i>B. flavum</i>) to enable modification of the methionine biosynthesis pathway, characterised in that it comprises the steps of (a) providing a</p> | | | |

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.

PCT/FR2004/000354

Box No. V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

modified microorganism by genetically modifying the cells of a starting microorganism so as to inhibit the production of a metabolite (homoserine) when the microorganism is cultured on a predetermined medium, whereby the growth capacity of the microorganism is adversely affected; (b) culturing the previously modified microorganisms obtained on said medium defined to cause evolution thereof (the medium contains glucose, soybean hydrolysate and inorganic nutrients, and the co-substrate enabling evolution is methyl mercaptan or H₂S); and (c) selecting cells having modified microorganisms capable of developing on the predetermined medium with the co-substrate. The method comprises an additional step (a1) of inserting at least one heterologous gene coding for a heterologous protein, which heterologous gene is intended to enable the evolution of a new metabolic pathway prior to step (b), i.e. a step of inserting genes coding for cystathione gamma-synthase and O-acyl-L-homoserine sulfhydrolase. Protein evolution enables the inhibited metabolic pathway (homoserine) to be replaced by a new metabolic pathway (methionine). It follows that the subject matter of claims 1 to 4 and 7 to 14 is anticipated by document D1. Furthermore, D1 shows such a gene coding for such a modified protein having "methionine synthase" activity and selected from cystathione gamma-synthases and O-acyl-L-homoserine sulfhydrolases, as well as the use of such a microorganisms or such a protein in a biotransformation method, i.e.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.

PCT/FR2004/000354

Box No. V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

the preparation of methionine. Consequently, the subject matter of claims 15 to 17 and 35 is anticipated by document D1.

2.2 The terms "modified" "modification(s)", "evolved" and "corresponding", as used in the claims, are vague, undefined and equivocal and thus cast doubt on the meaning of the technical features to which they refer and on the scope of the claims. It follows that the subject matter of the claims has not been clearly defined (PCT Article 6), and that the novelty of the claims is affected.

2.3 The indication "K183" does not appear to add an essential technical feature to the definition of claim 12.

2.4 Claims 18 to 25, 27 and 28 do not contain any features which, when combined with the features of any one of the claims to which they refer, comply with the requirements of novelty and inventive step of the PCT (PCT Article 33(2) and (3)). Document D2 (cf. the whole document) shows an "unmodified" cystathione gamma-synthase that is 100 % identical to the cystathione gamma-synthase sequence of E. coli K12 shown in SEQ ID NO 6. Document D3 (cf. the whole document) shows an "evolved" or "modified" enzyme including the amino acid sequence AASLGGVES in the C-terminal portion thereof, which sequence "matches" residues 324 to 332 of the sequence of E. coli cystathione gamma-synthase shown in SEQ ID NO 8.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.

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| Box No. V | Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement |
|-----------|---|
|-----------|---|

2.5 The combination of features in claim 26 is not found in or obvious from the prior art because it is not obvious for a person skilled in the art to arrive at a cystathione gamma-synthase having "methionine synthase" activity and including the amino acid sequence shown in SEQ ID NO 8.

2.6 The present application fails to comply with the requirements of PCT Article 33(1) since the subject matter of claims 1 to 4, 7 to 25, 27, 28 and 35 does not meet the requirement of novelty defined in PCT Article 33(2) and does not involve an inventive step as defined in PCT Article 33(3).

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.

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Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:
- a. type of material
 - ☒ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material
 - ☒ in written format
 - ☒ in computer readable form
 - c. time of filing/furnishing
 - ☒ contained in the international application as filed
 - ☒ filed together with the international application in computer readable form
 - ☐ furnished subsequently to this Authority for the purposes of search and/or examination
 - ☐ received by this Authority as an amendment* on _____
2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:
- The sequence listing in the description, pages 1-14, as originally filed

* If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

Box III

1. Claims: 1-4, 7-35 (in part, where applicable), 5, 6, 36, 37 (in full) (cf. Box IV: Lack of unity of invention: invention 1):

A method for preparing evolved microorganisms to enable modification of a methionine biosynthesis pathway, microorganisms, genes and proteins obtained by means of said method, and the use thereof in a biotransformation method.

2. The present claims 11 to 35 relate to products defined by reference to a desirable property or characteristic, namely the method by means of which they can be prepared and/or the fact that the enzyme in question has "modified methionine synthase" activity.

The claims cover all of the products that have this property or characteristic, whereas the application provides support (PCT Article 6) and disclosure (PCT Article 5) for only a very limited number of such products. In the present case, the claims lack support and the application lacks disclosure to such an extent that it is impossible to carry out a meaningful search covering the entire range of protection sought. Independently of the reasons given above, the claims also lack clarity (PCT Article 6). Indeed, an attempt has been made to define the product in terms of the

Supplemental Box

method by means of which they can be prepared and/or the fact that the enzyme in question has "modified methionine synthase" activity. This lack of clarity is, again, such that it is impossible to carry out a meaningful search covering the entire range of protection sought. Therefore, the search was directed only to the parts of the claims of which the subject matter appears to be clear, supported and sufficiently disclosed, namely the parts that relate to cystathione gamma-synthase mutation E325A (cf. claims 25 and 26) and clone K183 (cf. claim 12).

Supplemental Box

Box IV

The various groups of inventions are as follows:

1. Claims 1 to 4 and 7 to 35 (in part, where applicable).

A method for preparing evolved microorganisms to enable modification of a methionine biosynthesis pathway, microorganisms, genes and proteins obtained by means of said method, and the use thereof in a biotransformation method.

2. Claims 1 to 4 and 7 to 35 (in part, where applicable).

A method for preparing evolved microorganisms to enable modification of a cysteine biosynthesis pathway, microorganisms, genes and proteins obtained by means of said method, and the use thereof in a biotransformation method.

3. Claims 1 to 4 and 7 to 35 (in part, where applicable).

A method for preparing evolved microorganisms to enable modification of a threonine biosynthesis pathway, microorganisms, genes and proteins obtained by means of said method, and the use thereof in a biotransformation method.

Supplemental Box

4. Claims 1 to 4 and 7 to 35 (in part, where applicable).

A method for preparing evolved microorganisms to enable modification of a lysine biosynthesis pathway, microorganisms, genes and proteins obtained by means of said method, and the use thereof in a biotransformation method.

5. Claims 1 to 4 and 7 to 35 (in part, where applicable).

A method for preparing evolved microorganisms to enable modification of a isoleucine biosynthesis pathway, microorganisms, genes and proteins obtained by means of said method, and the use thereof in a biotransformation method.

6. Claims 1, 2 and 7 to 35 (in part, where applicable).

A method for preparing evolved microorganisms to enable modification of nucleic acid biosynthesis pathways, microorganisms, genes and proteins obtained by means of said method, and the use thereof in a biotransformation method.

7. Claims 1, 2 and 7 to 35 (in part, where applicable).

A method for preparing evolved microorganisms to enable modification of lipid biosynthesis

Supplemental Box

pathways, microorganisms, genes and proteins obtained by means of said method, and the use thereof in a biotransformation method.

8. Claims 1, 2 and 7 to 35 (in part, where applicable).

A method for preparing evolved microorganisms to enable modification of sugar biosynthesis pathways, microorganisms, genes and proteins obtained by means of said method, and the use thereof in a biotransformation method.

9. Claims 1, 2 and 7 to 35 (in part, where applicable); 5, 6, 36, 37 (in full).

A method for preparing evolved microorganisms to enable modification of the metabolic pathways involved in NADPH consumption, microorganisms, genes and proteins obtained by means of said method, and the use thereof in a biotransformation method.

The above inventions are not so linked as to form a single general inventive concept (PCT Rule 13.1), for the following reasons:

The prior art describes methods for preparing evolved microorganisms to enable modification of metabolic pathways (e.g. nucleic acid biosynthesis pathways and methionine biosynthesis), including the three steps described in claim 1. Document WO

Supplemental Box

02/083892 (cf. claims 1 to 30) describes an artificial *in vivo* protein evolution method whereby a protein X (e.g. a kinase) can be evolved by complementation of a related protein Y. the mutant protein X has a broader activity than the starting protein (for example, mutants D133E and R104Q of deoxycytidine kinase (DCK) have been obtained, and each of these mutations confers the acquisition of thymidine kinase activity by DCK). Document XP002154849 (cf. the whole document) describes a method for preparing evolved microorganisms including the three steps described in claim 1. Mutant hydantoinase has a reversed enantioselectivity and can be used in the an improved method for producing L-methionine.

In the light of the prior art, the problem addressed by the present application is that of providing alternative methods for preparing evolved microorganisms to enable alternative modification of metabolic pathways, including the three steps described in claim 1. Solutions 1 to 9 to said problem amount to providing methods for preparing evolved microorganisms to enable modification of a metabolic pathway relating to:

- (1) methionine biosynthesis;
- (2) cysteine biosynthesis;
- (3) threonine biosynthesis;
- (4) lysine biosynthesis;
- (5) isoleucine biosynthesis;
- (6) nucleic acid biosynthesis;
- (7) lipid biosynthesis;

Supplemental Box

(8) sugar biosynthesis;

(9) NADPH consumption.

Given that the methods for preparing evolved microorganisms to enable alternative modification of metabolic pathways, including the three steps described in claim 1, are described in the prior art (cf. WO 02/83892 and XP002154849), as a result of the essential technical differences between said solutions, and in view of the fact that it has been impossible to determine any other feature which might be considered to be a special technical feature in the light of the prior art the Search Division is of the opinion that no single general inventive concept covers the plurality of solutions proposed in the present application. It follows that the required unity of invention does not exist (PCT Rule 13.1) and since the various inventions do not have a common inventive concept, they are drafted as separate subjects as well as notified (PCT Article 17(3)(a)).